510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY TEMPLATE

A. 510(k) Number:
k062285

B. Purpose for Submission:
New product

C. Measurand:
Benzodiazepines

D. Type of Test:
Semi-quantitative and qualitative homogeneous enzyme immunoassay

E. Applicant:
Ortho-Clinical Diagnostic

F. Proprietary and Established Names:
VITROS Chemistry Products BENZ Reagent
VITROS Chemistry Products Calibrator Kit 26
VITROS Chemistry Products FS Calibrator 1
VITROS Chemistry Products DAT Performance Verifiers I. II, III, IV, and V

G. Regulatory Information:

1. Regulation section:
   21 CFR §862.3170, Benzodiazepine Test System
   21 CFR §862.3200, Clinical Toxicology Calibrator
   21 CFR §862.3280, Clinical Toxicology Control Material
2. Classification:
   Class II (Reagent, Calibrator)
   Class I Reserved (Control)
3. Product code:
   JXM; DKB; DIF
4. Panel:
   Toxicology (91)

H. Intended Use:

1. Intended use(s):
   See Indications for Use below.
2. Indication(s) for use:
   VITROS Chemistry Products BENZ Reagent is used on VITROS 5,1 FS Chemistry Systems for the semi-quantitative or qualitative determination of benzodiazepines (BENZ) in human urine using a cutoff of either 200 ng/mL or 300 ng/mL. Measurements obtained with the VITROS BENZ method are used in the diagnosis and treatment of benzodiazepines use or overdose.

The VITROS Chemistry Products BENZ assay is intended for use by professional laboratory personnel. It provides only a preliminary test result. A more specific alternative chemical method must be used to confirm a result obtained with this assay. Gas chromatography/mass spectrometry (GC/MS) is the preferred confirmatory method. Clinical consideration and professional judgment should be applied to any drug-of-abuse test result, particularly when evaluating a preliminary positive result.
VITROS Chemistry Products Calibrator Kit 26 is used to calibrate VITROS 5.1 FS Chemistry Systems for the qualitative or semi-quantitative measurement of the drugs of abuse.

VITROS Chemistry Products FS Calibrator 1: For in vitro diagnostic use only. VITROS Chemistry Products FS Calibrator 1 is used in conjunction with VITROS Chemistry Products Calibrator Kits to calibrate VITROS 5,1 FS Chemistry Systems

VITROS Chemistry Products DAT Performance Verifiers are assayed control used to monitor performance of urine drugs of abuse screening assays on VITROS 5,1 FS Chemistry Systems.

3. Special conditions for use statement(s):
   This device is for use by professional laboratory personnel. For *in vitro* diagnostic use only.

4. Special instrument requirements:
   Ortho-Clinical Diagnostics VITROS 5,1 FS Chemistry System

I. Device Description:

The VITROS BENZ Reagent is a dual-chambered package containing ready-to-use liquid reagents that are used to detect benzodiazepines in urine. Sample, calibrators, and controls are automatically treated with surfactant (DAT Diluent 2) prior to addition of reagents. Treated sample is added to Reagent 1 containing antibody reactive to diazepam, glucose-6-phosphate and nicotinamide adenine dinucleotide (NAD^+), followed by Reagent 2 containing diazepam labeled with the enzyme glucose-6-phosphate dehydrogenase (G6P-DH).

VITROS Chemistry Products Calibrator Kit 26 is prepared from human urine to which drugs of abuse, metabolites of drugs of abuse, organic salts, surfactants and preservative have been added.
VITROS Chemistry Products FS Calibrator 1 is prepared from sodium chloride and processed water. These products are used to calibrate VITROS 5,1 FS Chemistry Systems for the qualitative and semi-quantitative measurement of cocaine.
VITROS DAT Performance Verifiers I, II, III, IV & V are prepared from a human urine pool to which analytes, surfactant and preservative have been added. These are assayed controls used to monitor performance of the VITROS COCM Reagent on VITROS 5,1 FS Chemistry Systems.
The product labeling for the Calibrator Kit 26 and Performance Verifiers contain warnings regarding the presence of human sourced materials and recommend the use

of Universal Precautions when handling these products.

J. Substantial Equivalence Information:

1. Predicate device name(s):
   EMIT II Plus Benzodiazepine Assay
   Liquicheck Urine Toxicology Controls
2. Predicate 510(k) number(s):
   k993985; k022707
3. Comparison with predicate:

Similarities		
Item	Device	Predicate
Intended Use	For use in the qualitative and semi-quantitative analysis of benzodiazepines in human urine.	Same
Reagent	Liquid, ready to use	Same
Principle	Homogeneous enzyme immunoassay	Same
Matrix	Urine	Same
Antibody	Sheep polyclonal	Same

Differences		
Item	Device	Predicate
Instrumentation	VITROS 5,1 FS Chemistry Systems	Multiple automated clinical chemistry analyzers
Calibrators: Number of level	Six	Qualitative: Three at each cutoff value Semi-quantitative: Five
Controls: Number of levels	Five	Two

K. Standard/Guidance Document Referenced (if applicable):

CSLI EP9-A2: Method Comparison and Bias Estimation Using Patient Samples
CLSI EP5-A: Evaluation of Precision Performance of Clinical Chemistry Devices
CLSI EP6-A: Evaluation of the Linearity of Quantitative Measurement Procedures, A Statistical Approach
CLSI EP7-P: Interference Testing in Clinical Chemistry
CLSI EP17-A: Protocols for Demonstration, Verification and Evaluation of Limits of Detection and Quantitation
CLSI EP12-A: User Protocols for Evaluation of Qualitative Test Performance

L. Test Principle:

The VITROS BENZ assay is a homogeneous enzyme immunoassay that is performed using the VITROS Chemistry Products BENZ Reagent with the VITROS Chemistry

Products Calibrator Kit 26, VITROS Chemistry Products FS Calibrator 1, and VITROS Chemistry Products FS Diluent Pack 4 (DAT Diluent/DAT Diluent 2) on VITROS 5,1 FS Chemistry Systems.
The assay is based on competition between benzodiazepines in the treated urine sample and diazepam labeled with the enzyme glucose-6-phosphate dehydrogenase (G6P-DH) for antibody binding sites. Enzyme activity decreases upon binding to the antibody, therefore the concentration of benzodiazepines in the urine sample is directly proportional to measured enzyme activity. Active enzyme converts oxidized nicotinamide adenine dinucleotide (NAD^+) to NADH, resulting in an absorbance change that is measured spectrophotometrically at 340 nm.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

Imprecision was evaluated with human urine based quality control materials on the VITROS 5,1 FS Chemistry System following CLSI Protocol EP5 and CLSI protocol EP12.

Imprecision for BENZ: Semi-Quantitative

System	Conventional Units (ng/mL) and SI Units (µg/L)			Within Lab CV%**	No. Observ.	No. Days
	Mean Conc.	Within Day SD*	Within Lab SD**			
VITROS 5,1 FS	157	8.3	15.1	9.6	84	22
	233	10.1	20.5	8.8	84	22
	264	12.8	19.1	7.2	82	22
	397	17.2	31.3	7.9	82	22
	626	37.2	58.0	9.3	84	22

* Within Day imprecision was determined using one or two runs per day with two replicates per run.
** Within Lab imprecision was determined using a single lot of reagents with one analyzer and four calibrations.

Qualitative imprecision was assessed using test fluids targeted at ± 25% of each cutoff.

System	Cutoff Level (ng/mL & □g/L)	Test Fluid at ± 25% Cutoff	Number of Observations	Number of Correct Results
VITROS 5,1 FS	200	-25%	84	84
	200	+25%	82	82
	300	-25%	84	84
	300	+25%	82	82

b. Linearity/assay reportable range:

The sponsor followed CLSI EP6-A in determining the linear range of their device. The low and high concentration pools were mixed to give 13 admixtures of intermediate BENZ concentrations. Three determinations of all pools were made together with three determinations each of VITROS Chemistry Products

DAT Performance Verifiers I, II, III, IV, and V. This experiment was performed three times, once with each of three VITROS BENZ Reagent lots on the VITROS 5,1 FS Chemistry System. A linear regression was performed and the results indicated acceptable linearity across the range of 33 to 869 ng/mL. This linearity determination in conjunction with determination of the limit of quantitation was used to establish the reportable range of the VITROS BENZ assay to be 85 to 800 ng/mL.

Analytical Recovery of Semi-Quantitative Results

Eight admixtures were prepared from two human urine pools. The BENZ values for the admixtures were verified by GC/MS. Percent recovery was calculated using the concentration obtained by the VITROS Chemistry Products BENZ Assay versus the GC/MS value.

Recovery of Lormetazepam

GC/MS (ng/mL)	VITROS BENZ Assay (ng/mL)	% Recovery
661	642	97.1
557	539	96.7
465	454	97.6
364	378	103.9
281	288	102.4
190	199	104.9
142	158	111.0
101	99	97.8

c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*

The assigned values for the calibrators and controls are traceable to the Cerilliant lormetazepam standard catalogue L-910 and are verified by GC/MS. Real time and accelerated stability studies were conducted; protocols and acceptance criteria were described and found to be acceptable. These studies support the manufacturer's stability claims. Real time studies are ongoing.

d. *Detection limit:*

The detection limit was determined according to protocol recommendations in CLSI EP-17 on three different lots of reagent and one instrument platform. The claimed lower limit for VITROS BENZ is 85 ng/mL.

e. *Analytical specificity:*

The specificity of the VITROS BENZ assay for various benzodiazepines and structurally similar compounds was estimated by generating a dose response curve for each of the compounds listed below. The quantity (ng/mL) of compound that produces a value equivalent to the lormetazepam quantity (ng/mL) at each cutoff value is listed below. The combined effects of more than one compound detected in a sample may cause levels lower than those listed below to produce a value approximately equivalent to or greater than the cutoff value.

Substances that Cross-react with BENZ

Compound	Quantity (ng/mL) equivalent to 200 ng/mL of lormetazepam	% cross-reactivity *	Quantity (ng/mL) equivalent to 300 ng/mL of lormetazepam	% cross-reactivity *
nordiazepam	70	285.7	100	300.0
prazepam	90	222.2	120	250.0
tetrazepam	105	190.5	138	217.4
ketazolam	108	185.2	142	211.3
diazepam	118	169.5	160	187.5
N-desalkyflurazepam	120	166.7	182	164.8
medazepam	120	166.7	182	164.8
a-hydroxyalprazolam	120	166.7	155	193.5
flurazepam	130	153.8	171	175.4
a-hydroxytriazolam	140	142.9	185	162.2
nitrazepam	145	137.9	210	142.9
flunitrazepam	152	131.6	215	139.5
alprazolam	155	129.0	200	150.0
temazepam	165	121.2	230	130.4
norfludiazepam	195	102.6	260	115.4
halazepam	209	95.7	274	109.5
clonazepam	224	89.3	408	73.5
clobazam	240	83.3	440	68.2
oxazepam	270	74.1	380	78.9
7-aminoflunitrazepam	300	66.7	650	46.2
N-desmethyldiazepam	300	66.7	490	61.2
demoxepam	400	50.0	550	54.5
bromazepam	422	47.4	749	40.1
chlordiazeopoxide	1230	16.3	2900	10.3
7-aminoclonazepam	1800	11.1	4000	7.5
norchlordiazepoxide	1900	10.5	3750	8.0
oxazepam glucuronide	>10,000	<2	>10,000	<3
lorazepam glucuronide	>10,000	<2	>10,000	<3

* The VITROS BENZ Assay cutoff value (ng/mL) divided by the amount of cross-reactant (ng/mL) that produces a value equivalent to the cutoff value, multiplied by 100.

Interfering Substances:

Known Interfering Substances for BENZ

Cutoff Value (ng/mL)	Interferent*	Interferent Concentration		Bias (ng/mL)**
200	desipramine	10 mg/dL	330 µmol/L	+57
	dextromethorphan	10 mg/dL	368 µmol/L	+47
	dicyclomine	10 mg/dL	289 µmol/L	+47
	diethylpropion	10 mg/dL	487 µmol/L	+61
	ethacrynic acid	10 mg/dL	330 µmol/L	+73
	imipramine	10 mg/dL	357 µmol/L	+88
	indomethacin	2.5 mg/dL	70 µmol/L	+65

Known Interfering Substances for BENZ

Cutoff Value (ng/mL)	Interferent*	Interferent Concentration		Bias (ng/mL)**
	phenyltoloxamine	2.5 mg/dL	98 µmol/L	+76
	phenylbutazone	0.5 mg/dL	324 µmol/L	+56
	promethazine	2.5 mg/dL	20 µmol/L	+49
	sertraline	0.5 mg/dL	16 µmol/L	+51
	tripelannamine	1.0 mg/dL	39 µmol/L	+68
	tripolidine	10 mg/dL	359 µmol/L	+41
	NaCl	1500 mg/dL	207 mmol/L	+46
300	desipramine	10 mg/dL	330 µmol/L	+67
	dextromethorphan	10 mg/dL	368 µmol/L	+61
	dicyclomine	10 mg/dL	379 µmol/L	+75
	diethylpropion	10 mg/dL	487 µmol/L	+61
	doxylamine	10 mg/dL	370 µmol/L	+80
	ethacrynic acid	10 mg/dL	330 µmol/L	+80
	imipramine	10 mg/dL	357 µmol/L	+107
	indomethacin	5.0 mg/dL	140 µmol/L	+72
	phenyltoloxamine	2.5 mg/dL	98 µmol/L	+63
	phenylbutazone	10 mg/dL	324 µmol/L	+70
	sertraline	10 mg/dL	327 µmol/L	+152
	tripelannamine	1.0 mg/dL	39 µmol/L	+64

* The degree of interference at concentrations other than those listed might not be predictable from these results. Other interfering substances may be encountered in the patient population.

** The bias is an estimate of the maximum difference observed.

f. Assay cut-off:

The stated cutoffs of this assay are either 200ng/mL or 300 ng/mL.

2. Comparison studies:

a. Method comparison with predicate device:

A total of 115 human urine samples were assayed using the VITROS Chemistry Products BENZ Reagent and GC/MS or LC/MS reference method for lormetazepam. Percent agreement was evaluated at assay cutoff values of 200 ng/mL and 300ng/mL.

GC/MS Reference Method Comparison for BENZ

		GC/MS or LC/MS Reference				%Agreement		
Cutoff Value (ng/mL)		Low Negative	Near Cutoff Negative	Near Cutoff Positive	High Positive	%Agreement Negative	%Agreement Positive	%Agreement Overall
		(<-50%) <100 ng/mL	(-50% to cutoff) 100-200 ng/mL	(cutoff to +50%) 200-300 ng/mL	(>+50%) >300 ng/mL			
200	**VITROS Positive**	7	9	10	33	76.8	93.7	83.5
	VITROS Negative	52	3	0	1			

GC/MS Reference Method Comparison for BENZ

		GC/MS or LC/MS Reference				%Agreement		
Cutoff Value (ng/mL)		**Low Negative**	**Near Cutoff Negative**	**Near Cutoff Positive**	**High Positive**	**%Agreement Negative**	**%Agreement Positive**	**%Agreement Overall**
		(<-50%) <150 ng/mL	(-50% to cutoff) 150-300 ng/mL	(cutoff to +50%) 300-450 ng/mL	(>+50%) >450 ng/mL			
300	**VITROS Positive**	6	5	8	24	86.1	88.9	87.0
	VITROS Negative	55	13	3	1			

Summary of Discordant Results: GC/MS or LC/MS

Cutoff Value (ng/mL)	VITROS BENZ Assay (ng/mL)	Reference (ng/mL)
200	87	415 oxazepam & 26 temazepam on LC/MS
	108	395 alprazolam, 21 alpha-OH-alpraolam, and 168 7-amino-clonazepam on LC/MS
	173	184 alprazolam & 5 alpha-OH-alprazolam on LC/MS
	209	237oxazepam on LC/MS
	210	284 7-amino-clonazepam on LC/MS
	216	101 alpha-OH-alprazolam & 41 alprazolam on LC/MS
	224	303 7-amino-clonazepam on LC/MS
	243	90 oxazepam, 26 temazepam, & 4 nordiazepam on LC/MS
	248	38 alprazolam and 64 alpha-OH-alprazolam on LC/MS
	265	155 oxazepam, 23 temazepam, & 8 nordiazepam on LC/MS
	303	284 7-amino-clonazepam on LC/MS
	303	343 7-amino-clonazepam on LC/MS
	319	106 alpha-OH-alprazolam on GC/MS
	342	90 oxazepam, 20 temazepam, & 15 nordiazepam on LC/MS
	422	26 oxazepam & 6 of alpha-OH-alprazolam on LC/MS
	>RR	237 oxazepam on GC/MS
	>RR	71 alprazolam and 51 alpha-OH-alprazolam on LC/MS
	>RR	< 75 on GC/MS
	>RR	76 oxazepam on GC/MS

Summary of Discordant Results: GC/MS or LC/MS

Cutoff Value (ng/mL)	VITROS BENZ Assay (ng/mL)	Reference (ng/mL)
300	87	415 oxazepam &26 temazepam on LC/MS
	108	395 alprazolam, 21 alpha-OH-alpraolam, and 168 7-amino-clonazepam on LC/MS
	250	200 temazepam, 104 oxazepam, & 42 nordiazepam on LC/MS
	277	169 oxazepam, 127 temazepam, & 50 nordiazepam on LC/MS
	303	284 7-amino-clonazepam on LC/MS
	303	343 7-amino-clonazepam on LC/MS
	319	106 alpha-OH-alprazolam on GC/MS
	342	211 oxazepam, 31 temazepam, & 15 nordiazepam on LC/MS
	342	90 oxazepam, 20 temazepam, & 15 nordiazepam on LC/MS
	422	26 oxazepam & 6 of alpha-OH-alprazolam on LC/MS
	466	118 alpha-OH-alprazolam & 38 alprazolam on LC/MS
	>RR	< 75 on GC/MS
	>RR	76 oxazepam on GC/MS
	>RR	237 oxazepam on GC/MS
	>RR	71 alprazolam and 51 alpha-OH-alprazolam on LC/MS

A total of 115 human urine samples were assayed using the VITROS Chemistry Products BENZ Reagent and a commercially available immunoassay method, for benzodiazepine. Percent agreement was evaluated at assay cutoff values of 200 ng/mL and 300 ng/mL.
To challenge performance at the 200 ng/mL cutoff value, 44 of the 115 samples tested had concentrations within +/- 50% of the cutoff value, 27 samples below the cutoff value and 17 above the cutoff value.
To challenge performance at the 300 ng/mL cutoff value, 39 of the 115 samples tested had concentrations within +/- 50% of the cutoff value, 30 samples below the cutoff value and 9 above the cutoff value.

Commercial Method Comparison for BENZ

		Commercial Method**				%Agreement		
Cutoff Value (ng/mL)		**Low Negative**	**Near Cutoff Negative**	**Near Cutoff Positive**	**High Positive**	**%Agreement Negative**	**%Agreement Positive**	**%Agreement Overall**
200		(<-50%) <100 ng/mL	(-50% to cutoff) 100-200 ng/mL	(cutoff to +50%) 200-300 ng/mL	(>+50%) >300 ng/mL	100.0	95.2	97.4
	VITROS Positive	0	0	14	45			
	VITROS Negative	26	27	3*	0			
300		(<-50%) <150 ng/mL	(-50% to cutoff) 150-300 ng/mL	(cutoff to +50%) 300-450 ng/mL	(>+50%) >450 ng/mL	98.6	93.3	96.5
	VITROS Positive	0	1*	6	36			
	VITROS Negative	40	29	3*	0			

b. Matrix comparison:
Not applicable; this device is for use with urine only.

3. Clinical studies:
   a. Clinical Sensitivity:
   Not applicable.
   b. Clinical specificity:
   Not applicable.
   c. Other clinical supportive data (when a. and b. are not applicable):
   Not applicable
4. Clinical cut-off:
   Not applicable.
5. Expected values/Reference range:
   Not applicable.

N. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

O. Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.